REMARKS

Claims 5, 13, 17, and 19-24 have been amended and claims 6-12 and 25 have been cancelled. Claims 5 and 13-24 are currently pending in the application. All pending claims are set forth in Exhibit A with amendments shown (if applicable).

Applicants gratefully acknowledge the 14 February 2003 interview with the Examiner in which all pending rejections in the application were discussed. During the interview, the Examiner agreed that the present amendments have addressed the concerns raised over new matter.

Applicants note in claims 5 and 19 the term "sensitizer"in reference to the second reagent has been removed in response to the Examiner's concern.

Basis for the amendments are as follows:

Claim(s)	Term/Phrase	Basis
5, 13, 17, 19, 23	"target-binding moiety"	Page 7, line 36-38; Page 10, lines 3-4; Page 31, lines 21-22.
5, 19	"selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydrogen radicals" in reference to active species.	Page 18, line 27, of parent application 09/698,846.*
19	"comprising a detection group, D, and a mobility modifier, M,"	Page 4, lines 19-22.
19-24	"specific binding pairs"	Page 31, lines 9-20. Table 4 on page 33.
5, 17, 19, 24	"second reagent"	Page 18, line 22, of parent application 09/698,846*

* Parent application 09/698,846 was incorporated by reference (see page 1, lines 5-8, of the specification), and the indicated passages were expressly incorporated by the 22 July 2002 Amendment.

No new matter has been added by the amendments. Reconsideration is respectfully requested.

Rejections Under 35 U.S.C. 112 First Paragraph

In paragraph 4 of the Office Action, the Examiner rejected claims 5-25 under 35 U.S.C. 112 first paragraph because the following phrases in the claims were not found in the specification at the locations indicated in the prior amendment: (i) "antibody binding compound," (ii) "said cleaving agent is a sensitizer and said active species is singlet oxygen or hydrogen peroxide," and (iii) "said sensitizer is capable of generating singlet oxygen when photoactivated."

In view of the amendments, Applicants submit that the concerns raised by the Examiner over new matter have been addressed and respectfully request that the above rejection be withdrawn.

Rejections Under 35 U.S.C. 112 Second Paragraph

In paragraph 6 of the Office Action, the Examiner rejected claims 6-18 under 35 U.S.C. 112 second paragraph for the use of the term "complexes of electrophoretic probes," that allegedly has no antecedent basis.

Applicants respectfully disagree with this rejection, particularly in view of the amendments. The claims containing the phrase have been cancelled thereby obviating the Examiner's concern. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. 103

In paragraph 8 of the Office Action, the Examiner rejected claims 5-10, 12-14, 16-21, and 23 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski et al (U.S. patent 4,331,590) in view of Giese (Anal. Chem., 2: 166-168 (1983)). The Examiner argues as follows: Bocuslaski discloses ligands attached to indicator moieties each containing an enzymatically cleavable linkage, such that when the cleavable linkage is broken the indicator moiety becomes optically distinguishable from indicator moieties having intact linkages. Giese discloses sets of releasable electrophoric tags that may be attached to binding compounds and used in binding assays to detected multiple analytes in a single reaction. Released electrophoric tags are identified after separation by gas chromatography with electron capture detection. From Giese's teaching of the potential usefulness of multiplexed assays, one of ordinary skill in the art would have been motivated to combine probes having cleavable moieties of Giese or Bocuslaski to construct the kits of Applicants' invention.

Applicants respectfully disagree, particularly in view of the amendments. Applicants' kits differ from anything disclosed or suggested by Giese and/or Bocuslaski in at least two important respects: (1) Applicants' kits include a "second reagent" that can generate an active species selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydrogen radicals, and (2) the eTag reporters of Applicants' kits must be suitable for electrophoretic separation, whereas the "release" tags of Giese are designed for separation by gas chromatography (and no separation of molecular tags is taught by Bocuslaski).

In regard to (1), the "second reagent" of Applicants' kits is important for carrying out homogeneous assays, as briefly mentioned on page 31, line 18, of the specification, and as explained more fully in parent application 09/698,846, on page 18, line 16, to page 19, line 3. In accordance with

the homogeneous format of Applicants' invention, at least two types of reagents are provided: (A) electrophoretic probes that specifically bind to target compounds, and (B) a second reagent that specifically binds to the same targets in the proximity of an electrophoretic probe and that generates a short-lived active species for releasing the eTag reporters of the *proximate* electrophoretic probe. Neither Giese nor Bocuslaski, either alone or together, disclose or suggest a homogeneous assay of this type.

In Giese, homogeneous assays are not disclosed at all. Giese at best suggests only a *hetero*geneous assay format using a cleavage agent that *operates throughout an assay reaction* (cyanogen bromide cleavage, page 167, second column, lines 13-16, and Fig. 3). Thus, if Giese's "release" tags are attached to binding moieties, such as a monoclonal antibodies, a step must be implement to separate unbound monoclonal antibodies from those that specifically bind to a target. Thus, Giese actually teaches away from Applicants' kits that are used to implement homogeneous assays involving a second reagent for generating an *active species that operates only locally* to cleave proximate eTag reporters. In assays of Applicants' kits, unbound binding moieties DO NOT have to be separated from those that specifically bind to their targets.

In Bocuslaski, a homogeneous format is described in col. 10, lines 3-29, and schematically in Fig. 1; however, the principle of the assay and the nature of the assay readout are different from those in Applicants' assay. First, in the homogeneous format of Bocuslaski, the *cleavage agent (an enzyme) operates throughout the assay reaction.* A compound with a cleavage-activated label or indicator moiety (i.e. a "labeled compound") competes with the unlabeled analyte for binding to a third agent (e.g. an antibody) that protects the labeled compound from being cleaved (see section from col. 14, line 1, to col. 19, line 5 and the Examples). Thus, the higher the concentration of the analyte, the more labeled compound is available for cleavage by the enzyme and the higher the signal that is generated. In contrast, Applicants' homogeneous assay employs a second reagent that specifically binds to a target compound and generates *an active species that, in turn, releases eTag reporters from only proximate electrophoretic probes*.

In regard to (2)(electrophoretic separation v. gas chromatographic separation), Applicants submit that the gas phase separation of Giese limits the nature of the released tags that can be used and requires that additional steps be performed prior to separation.

In regard to the additional steps to implement Giese's method, for measurements in the disclosed binding assays, Giese's method requires that "release" tags be extracted from the assay mixture into a volatile organic solvent, then concentrated by evaporation prior to injection into a gas chromatograph (page 167, col. 2, last sentence in first full paragraph). This is a time consuming and

labor intensive step that is not required by Applicants' method. In accordance with the assay implemented by Applicants' kits, *released eTag reporters can be separated by electrophoresis directly from an assay reaction mixture.*

In regard to the nature of tags that can be separated by gas chromatography, Applicants direct the Examiner's attention to the following excerpt from a description of gas chromatography on the Agilent, Inc. website:

> "It is estimated that 10-20% of the known compounds can be analyzed by GC. To be suitable for GC analysis, a compound must have sufficient volatility and thermal stability. If all or some of a compound's molecules are in the gas or vapor phase at 400-450°C or below, *and they do not decompose at these temperatures*, the compound can probably be analyzed by GC."(www.chem.agilent.com/cag/cabu/whatisgc.htm) (copy attached as Exhibit B). (Emphasis added).

Many optically active molecules, such as organic fluorescent molecules, are not available for use in Giese's method because such molecules decompose rather than volatilize at temperatures necessary for gas chromatography (e.g. rhodamine B decomposes at 211°C, carboxyfluorescein decomposes at 275°C, and the like, data from Aldrich catalog). This is not a limitation in Applicants' invention because electrophoretic separation takes place in aqueous conditions that (by definition) must be less than boiling temperature, i.e. less than 100°C.

Applicants submit that neither Bocuslaski nor Giese diclose or suggest alone or in combination the electrophoretic separation of molecular tags, such as eTag reporters. In fact, Applicants submit that Giese teaches away from the combination with Bocuslaski because the latter reference teaches the use of fluorescent organic molecules as labels and this class of labels are generally not amenable to gas chromatographic analysis. (Applicants note that conventional detectors in gas chromatographs are based on thermal conductivity, flame ionization, electron capture, or mass spectrometry, e.g. Harris, "Exploring Chemical Analysis," 2nd edition (Freeman, San Francisco, 2001), none of which are suitable for organic fluorescent molecules.) Therefore, application of the multiplexing taught by Giese, which depends on gas chromatography, would not have been obvious to one of ordinary skill practicing the single-plex fluorescent labels of Bocuslaski.

For the above reasons, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

In paragraph 9 of the Office Action, the Examiner rejected claims 11 and 24-25 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski et al (U.S. patent 4,331,590) in view of Giese

(Anal. Chem., 2: 166-168 (1983)) as applied above, and further in view of Breslow et al (U.S. patent 6,331,530). Breslow discloses a compound for cancer therapy consisting of cyclodextrin dimers whose cyclodextrin pairs are connected by a linkage which is cleaved by singlet oxygen generated by a photosensitizer. The purpose of Breslow's compound is to create a locally elevated concentration of photosensitizers in a region of illumination (for example, by a tumor). The Examiner applies Bocuslaski and Giese as above and further cites Breslow for its disclosure of a sensitizer used to generate the active species, singlet oxygen, for cleaving a chemical bond. The Examiner argues that one of ordinary skill in the art would have been motivated to employ the sensitizer-based cleavage system of Breslow because its use in cancer therapy suggests that it is highly efficient and would therefore be applicable to the analytical assays of Giese and Bocuslaski.

Applicants respectfully disagree, particularly in view of the amendments. Applicants submit that the combination of Giese and Bocuslaski are inappropriately applied as discussed above, and that the teachings of Breslow do not correct this deficiency with respect to claims 11 and 24-25. In fact, assays based on Bocuslaski and Giese as argued by the Examiner would more likely lead one of ordinary skill in the art away from Breslow because the photosensitizers of Breslow add complexity to an otherwise simple assay. For example, Giese teaches the use of cyanogens bromide for cleavage of his "release" tags and Bocuslaski teaches enzymatic cleavage of the indicator molecule. Both of these approaches involve the addition of only a single reagent. On the other hand, the system of Breslow requires (1) the addition of a photosensitizer, (2) a means for illuminating the photosensitizer, and (3) the selection of either an indicator molecule or a "release" tag that would not be affected by the chemically active singlet oxygen. Furthermore, the advantage of using the sensitizer-based cleavage taught by Breslow (enhance the localized tumor-killing affects of singlet oxygen—col. 3, lines 21-22) is not a factor that would motivate one of ordinary skill in the art to apply it to an analytical assay. Accordingly, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

The Examiner rejected claims 15 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski et al (U.S. patent 4,331,590) in view of Giese (Anal. Chem., 2: 166-168 (1983)) as applied above, and further in view of McGall et al (U.S. patent 5,843,655). McGall discloses a method for testing the efficiency of solid phase synthesis of oligonucleotides on microarrays. The method includes providing a substrate having a surface with cleavable linkers on which to carry out oligonucleotide synthesis. After synthesis is complete and a detectable label is coupled to the oligonucleotides, the labeled oligonucleotides are cleaved and their lengths are determined to provide a measure of how efficient the

synthesis was. A wide variety of conventional cleavable linkers are suggested, including those that are cleaved by oxidation. The Examiner argues that one of ordinary skill in the art would have been motivated to employ the cleavable linkages taught by McGall because the requirements for efficiency in McGall's system are equivalent to the requirements of an efficient cleavage to release tags or indicator molecules in the analytical assays of Giese and Bocuslaski. Thus, Giese and Bocuslaski together with McGall render the claim 15 embodiment of Applicants' invention obvious to one of ordinary skill in the art.

Applicants respectfully disagree, particularly in view of the amendments. Applicants submit that the combination of Giese and Bocuslaski are inappropriately applied as discussed above, and that the teachings of McGall do not correct this deficiency with respect to claim 15. In particular, the cleavable linkage of Applicants' invention is cleavable by an active species delivered by a second reagent. No such cleavage system is disclosed or suggested in any of the cited references either alone or in combination. Moreover, McGall actually teaches away from the use of such a system because the objectives of McGall's invention is quality control of a entirely man-made process (solid phase oligonucleotide synthesis) so that issues of homogeneity or heterogeneity of format are not important. In contrast, an important object of Appliants' kits is to permit a homogeneous assay for detecting one or more analytes. As described above, in order to achieve such an objective, a short-lived active species and specific binding pairs must be used. Applicants submit that none of these concepts are disclosed or suggested by any of the cited references; thus, one of ordinary skill in the art would not have been motivated to combine the teachings of McGall with those of Giese and Bocuslaskit to achieve Applicants' invention.

Accordingly, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

In view of the above, Applicants submit that the claims as written fully satisfy the requirements of Title 35 of the U.S. Code, and respectfully request that the rejections thereunder be withdrawn and that the claims be allowed and the application quickly passed to issue.

If any additional time extensions are required, such time extensions are hereby requested. If any additional fees not submitted with this response are required, please take such fees from deposit account **50-2266**.

Respectfully submitted,

Stephen C. Macevicz
Reg. No. 30,285
Attorney for Applicants

Telephone: (650) 210-1223
Email: smacevicz@aclara.com

Exhibit A
Claims showing Insertions and Deletions

5. (Amended) A kit for detecting the presence or absence of one or more target compounds, the kit comprising:

a plurality of electrophoretic probes each comprising target-binding moiety [~~an antibody binding compound~~] specific for a target compound, each target-binding moiety [~~an antibody binding compound~~] having one or more eTag reporters attached thereto by cleavable linkages such that upon cleavage of the cleavable linkages the eTag reporters from different electrophoretic probes form distinct peaks upon electrophoretic separation; and

a second reagent specific for at least one of said one or more target compounds, the second reagent being capable of generating an active species to cleave the cleavable linkage, the active species being selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydroxyl radicals.

6. (Cancelled) [~~The kit of claim 5 further including a capture agent for separating complexes of said electrophoretic probes specifically bound to said target compounds from unbound electrophoretic probes.~~]

7. (Cancelled) [~~The kit of claim 6 further including a cleaving agent for cleaving said eTag reporters from said electrophoretic probes of said complexes.~~]

8. (Cancelled) [~~The kit of claim 7 wherein said capture agent comprises a solid support having attached thereto antibody or antibody fragments that bind specifically to said one or more target compounds.~~]

9. (Cancelled) [~~The kit of claim 8 wherein said cleaving agent is an enzyme.~~]

10. (Cancelled) [~~The kit of claim 7 wherein said cleaving agent generates an active species for cleaving said cleavable linkage.~~]

11. (Cancelled) [~~The kit of claim 10 wherein said cleaving agent is a sensitizer and said active species is singlet oxygen or hydrogen peroxide.~~]

12. (Cancelled) [~~The kit of claim 5 further including a second antibody binding compound specific for at least one of said one or more target compounds, the second antibody binding compound having a sensitizer attached for generating an active species.~~]

13. (Amended) The kit according to claim 5 [~~any one of 5, 6, 7, 8, 9, 10, 11, or 12,~~] wherein said electrophoretic probes are selected from the group defined by the formula:

$$[(D, M)\text{-}L]_k\text{-}T$$

wherein:

T is a target-binding moiety [~~said antibody binding compound~~] specific for a target compound;

k is an integer in the range of from 1 to 20;

L is said cleavable linkage;

D is a detection group; and

M is a mobility modifier consisting of from 1 to 500 atoms selected from the group consisting of carbon, hydrogen, oxygen, sulfur, nitrogen, phosphorus, and boron.

14. The kit of claim 13 wherein said plurality is in the range of from 5 to 100, and wherein M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron.

15. The kit of claim 14 wherein said cleavable linkage is cleavable by oxidation and is selected from the group consisting of olefins, thioethers, sulfoxides, and selenium analogs of thioethers or sulfoxides.

16. The kit of claim 15 wherein said detection group is a fluorescent label.

17. (Amended) The kit of claim 16 wherein said target-binding moiety and said second reagent are each [~~antibody binding compound is~~] a monoclonal antibody or a polyclonal antibody; and wherein k is in the range of from 1 to 3.

18. The kit of claim 17 wherein said detection group is a fluorescein.

19. **(Amended)** A kit of [~~reagent~~] specific binding pairs for detecting the presence or absence of one or more target compounds, the kit comprising a plurality of pairs of first reagents and second reagents, the first reagent and second reagent of each pair being specific for the same target compound, the first reagent of each pair being selected from the group defined by the formula:

$$[(D, M)\text{-}L]_k\text{-}T$$

wherein:

T is a target-binding moiety [~~an antibody binding compound~~] specific for a target compound,

k is an integer in the range of from 1 to 20,

L is a cleavable linkage,

D is a detection group, and

M is a mobility modifier consisting of from 1 to 500 atoms selected from the group consisting of carbon, hydrogen, oxygen, sulfur, nitrogen, phosphorus, and boron,

wherein upon cleavage of L an eTag reporter comprising a detection group, D, and a mobility modifier, M, is produced with a distinct charge/mass ratio so that eTag reporters of different electrophoretic probes form distinct peaks upon electrophoretic separation; and

the second reagent of each pair [~~comprising a second antibody binding compound having a sensitizer for~~] being capable of generating an active species to cleave the cleavable linkage, the active species being selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydrogen radicals.

20. **(Amended)** The kit of [~~reagent~~] specific binding pairs of claim 19 wherein said plurality is in the range of from 5 to 100, and wherein M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron.

21. **(Amended)** The kit of [~~reagent~~] specific binding pairs of claim 20 wherein said cleavable linkage is selected from the group consisting of olefins, thioethers, sulfoxides, and selenium analogs of thioethers or sulfoxides.

22. (Amended) The kit of [~~reagent~~] specific binding pairs of claim 21 wherein said detection group is a fluorescent label, and wherein said charge/mass ratio is in the range from -.001 to 0.5.

23. (Amended) The kit of [~~reagent~~] specific binding pairs of claim 22 wherein said target-binding moiety [~~antibody binding compound~~] is a monoclonal antibody or a polyclonal antibody, and wherein k is in the range of from 1 to 3.

24. (Amended) The kit of [~~reagent~~] specific binding pairs according to claims 19, 20, 21, 22, or 23 wherein said second reagent [~~antibody binding compound~~] is a monoclonal antibody or a polyclonal antibody, and wherein said active species is singlet oxygen [~~or hydrogen peroxide~~].

25. (Cancelled) [~~The kit of reagent pairs of claim 35 wherein said sensitizer is capable of generating singlet oxygen when photoactivated.~~]